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L1        121508 S LIPOSOME#
L2       1787339 S ANTIBOD?
L3       1758451 S TUMOR#
L4         10936 S L1 AND L2
L5          1727 S L3 AND L4
L6           800 DUPLICATE REM L5 (927 DUPLICATES REMOVED)
L7        164117 S POLYETHYLENE (W) GLYCOL
L8            39 S L6 AND L7
L9            39 DUPLICATE REM L8 (0 DUPLICATES REMOVED)
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ANSWER 7 OF 39 CAPLUS COPYRIGHT 2002 ACS

TI Ligand-bonded complex

AB A ligand-bonded complex which can react not with free targets such as a sol. **tumor** antigen but substantially specifically with an unliberated target such as a **tumor** cell or a **tumor** antigen occurring in the cell.

SO PCT Int. Appl., 37 pp.
CODEN: PIXXD2

IN Tagawa, Toshiaki; Suzuki, Tsutomu; Yada, Nobuhisa; Nagaike, Kazuhiro; Hirakawa, Youko; Hosokawa, Saiko

L9 ANSWER 1 OF 39 CAPLUS COPYRIGHT 2002 ACS
TI Biopanning and rapid analysis of selective interactive ligands (BRASIL)
AB The present invention concerns novel methods of identifying peptide sequences that selectively bind to targets. In alternative embodiments, targets may comprise cells or clumps of cells, particles attached to chems. compds., mols. or aggregates, or parasites. In preferred embodiments, target cells are sorted before exposure to the phage library. The general method, Biopanning and Rapid Anal. of Selective Interactive Ligands (BRASIL) provides for rapid and efficient sepn. of phage that bind to targets, while preserving unbound phage. BRASIL may be used in preselection procedure to subtract phage that bind non-specifically to a first target before exposing the subtracted library to a second target. Certain embodiments concern targeting peptides identified by BRASIL and methods of use of such peptides for targeted delivery of therapeutic agents or imaging agents or diagnosis or treatment of diseases. Novel compns. comprising a first phase, second phase, target and a phage library are also disclosed. BASIL is exemplified by screening for targeting peptides for (1) VEGF in HUVEC cells, (2) the Molt-4 leukemia cell line, (3) urothelial tissue (human bladder wall), (4) mesenchymal stem cells, and (5) screening for bone marrow targeting peptides.
SO PCT Int. Appl., 167 pp.
CODEN: PIXXD2

L9 ANSWER 2 OF 39 CAPLUS COPYRIGHT 2002 ACS
TI Epitopes formed by non-covalent association of conjugates
AB A compn. for interacting with a ligand, which compn. comprises a non-covalent assocn. of a plurality of distinct conjugates, each conjugate comprising a head group and a tail group, wherein the tail groups of the conjugates form a hydrophobic aggregation and the conjugates are movable within the assocn. so that, in the presence of a ligand, at least two of the head groups are appropriately positioned to form an epitope capable of interacting with the ligand more strongly than each of head groups individually. The invention aims to overcome the problems involved in the development of protein receptor-specific therapeutic conjugates that includes evoking immune response or attacking by endopeptidases. The conjugates comprise a head group of amino acid, peptide, monosaccharide, polysaccharide, nucleotide, polynucleotide, sterol, water-sol. vitamin, porphyrin, metal ion chelate, water-sol. drug, hormone, enzyme substrate; a spacer of hydroxy acid, amino acid, sugar or **polyethylene glycol**; and a tail group of branched-chain fatty acid, alc., aldehyde, prostaglandin, leukotriene, glyceride, sphingosine, ceramide, silicon or deriv.
SO PCT Int. Appl., 39 pp.
CODEN: PIXXD2

L9 ANSWER 3 OF 39 CAPLUS COPYRIGHT 2002 ACS
TI Modified binding molecules specific for T lymphocytes and their use as in vivo immune modulators in animals
AB Several forms of immunoregulatory substances are derived from monoclonal **antibodies** (MAbs) that are specific for a T cell surface antigen, such as CD3, TCR, CD4, or CD8 on T cells. The substances include: a mixt. of F(ab')2 fragments (or other divalent binding mols. which lack Fc) which each bind noncompetitively to different monovalent antigenic epitopes on the same antigen; the F(ab')2 fragment (or other divalent binding mols. which lack Fc) of a bispecific **antibody** which has each of its binding sites derived from one of the two MAbs that bind noncompetitively to monovalent antigenic epitopes on the same antigen; a conjugate including a polymeric backbone, such as **polyethylene glycol** ("PEG"), cellulose, dextran, agarose, or an amino acid copolymer or a **liposome**, that is coupled with the binding mols., e.g., Fv, Fab, or F(ab')2, which bind noncompetitively to monovalent antigenic epitopes on the same antigen.
SO U.S., 9 pp., Cont.-in-part of U.S. 5,872,222.
CODEN: USXXAM

L9 ANSWER 4 OF 39 CAPLUS COPYRIGHT 2002 ACS

TI Anti-tissue factor **antibo**-chemotherapeutic agent conjuga**s**
AB The invention relates to an anti-tissue factor **antibody** -antitumor agent conjugate or an anti-tissue factor **antibody** -toxin conjugate with a linking agent providing improved drug targeting effect. An immunotoxin of anti-tissue factor **antibody**-gelonin conjugate was prepd. with N-succinimidyl 3-(2-pyridyldithio)propionate, and its inhibitory effect on protein synthesis in J 82 human bladder carcinoma cells was examd.
SO Jpn. Kokai Tokkyo Koho, 16 pp.
CODEN: JKXXAF

L9 ANSWER 5 OF 39 CAPLUS COPYRIGHT 2002 ACS
TI Study on third-type immunoliposomes loaded drugs and targeting in vitro and in vivo
AB The third-type immunoliposome (IML) loaded anticancer drugs- adriamycin (ADM) was prepd. from the conjugate of monoclonal **antibody** of human bladder cancer with PEG-COOH (**polyethylene glycol** carboxylic acid). The survival rate of the targeting EJ cells treated with IML- ADM (ADM = 45.45 .mu.g mL-) was 4.3 .+-. 1.0%, but 72% .+-. 6% for non-targeting LOVO cells in vitro. The **tumor** wt. in nude mice implanted by EJ cells was (39 .+-. 25), (135 .+-. 32), and (598 .+-. 240) mg by treatment with IML- ADM, SSL-ADM (steric stable **liposomes** carried Adriamycin), and normal saline for 27 d, resp. The results showed that the immunoliposme-mediated targeting anticancer drug was a feasible way.
SO Yaoxue Xuebao (2001), 36(7), 539-542
CODEN: YHHPAL; ISSN: 0513-4870

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L9 ANSWER 6 OF 39 CAPLUS COPYRIGHT 2002 ACS
TI Stealth monensin immunoliposomes as potentiator of immunotoxins in vitro
AB Stealth monensin **liposomes** (SML) were prepd. using dipalmitoyl phosphatidylcholine, cholesterol, distearoyl glycerophosphoethanolamine coupled to **polyethylene glycol**, stearylamine, and N-succinimidyl pyridodithiopropionate linked to stearyl amine, in the molar ratio of 10:5:1.4:1.4:1.5. SML was conjugated to the anti-MY9 **antibody** by a disulfide linkage to form stealth monensin immunoliposomes (SMIL) by an already established procedure. The encapsulation concns. of monensin in SML and SMIL were 10-7 and 4.9.times.10-8 M, resp. More than 20% of monensin remained in circulation after 24 h in BALB/c mice. The ability of SML and SMIL to potentiate the effect of anti-MY9 immunotoxin (anti-MY9-IT) was tested against human leukemia HL-60 sensitive and resistant **tumor** cells in vitro. SML and SMIL potentiated the activity of anti-MY9-IT by 10-20 times against HL-60 sensitive **tumor** cell lines. However, greater

potentiation of anti-MY9 was obsd. in combination with [redacted]L and SMIL against HL-60 resistant **tumor** cells, found to be 200 and 500 times, resp. The potentiation of anti-MY9-IT by SMIL was more than two-fold compared with SML against both HL-60 sensitive and resistant **tumor** cells. Transmission electron microscopy studies conducted with HL-60 resistant cells incubated with anti-MY9-IT and monensin **liposomes** showed significant dilation of the golgi, which was reversible after re-incubation in fresh medium. Our studies show that SML and SMIL can be successfully used to potentiate the activity of ricin based anti-MY9-IT in vitro, and further in vivo studies will demonstrate the usefulness of this approach.

SO Eur. J. Pharm. Biopharm. (2001), 52(1), 13-20
CODEN: EJPBEL; ISSN: 0939-6411

AU Singh, M.; Ferdous, A. J.; Kanikkannan, N.; Faulkner, G.

L9 ANSWER 7 OF 39 CAPLUS COPYRIGHT 2002 ACS

TI Ligand-bonded complex

AB A ligand-bonded complex which can react not with free targets such as a sol. **tumor** antigen but substantially specifically with an unliberated target such as a **tumor** cell or a **tumor** antigen occurring in the cell.

SO PCT Int. Appl., 37 pp.
CODEN: PIXXD2

IN Tagawa, Toshiaki; Suzuki, Tsutomu; Yada, Nobuhisa; Nagaike, Kazuhiro; Hirakawa, Youko; Hosokawa, Saiko

L9 ANSWER 8 OF 39 CAPLUS COPYRIGHT 2002 ACS

TI Novel methods of imaging and treatment with targeted compositions

AB Novel ultrasound methods comprising administering to a patient a targeted vesicle compn. which comprises vesicles comprising a lipid, protein or polymer, encapsulating a gas, in combination with a targeting ligand, and scanning the patient using ultrasound. The scanning may comprise exposing the patient to a first type of ultrasound energy and then interrogating the patient using a second type of ultrasound energy. The targeting ligand preferably targets tissues, cells or receptors, including myocardial cells, endothelial cells, epithelial cells, **tumor** cells and the glycoprotein GPIIbIIIa receptor. The methods may be used to detect a thrombus, enhancement of an old or echo genic thrombus low concns. of vesicles and vesicles targeted to tissues, cells or receptors.

SO PCT Int. Appl., 211 pp.
CODEN: PIXXD2

IN Ungr, Evan C.; Wu, Yunqiu

L9 ANSWER 9 OF 39 CAPLUS COPYRIGHT 2002 ACS

TI Modified binding molecules specific for T or B lymphocytes and their use as in vivo immune modulators

AB Several forms of immunoregulatory substances are derived from monoclonal **antibodies** (MAbs) that are specific for a T or B cell surface antigen, such as CD3, TCR, CD4, or CD8 on T cells or membrane-bound Igs on B cells. The substances include: a mixt. of F(ab')2 fragments (or other divalent binding mols. which lack Fc) which each bind noncompetitively to different monovalent antigenic epitopes on the same antigen; the F(ab')2 fragment (or other divalent binding mols. which lack Fc) of a bispecific **antibody** which has each of its binding sites derived from one of the two MAbs that bind noncompetitively to monovalent antigenic epitopes on the same antigen; a conjugate including a polymeric backbone, such as **polyethylene glycol** ("PEG"), cellulose, dextran, agarose, or an amino acid copolymer or a **liposome**, that is coupled with the binding mols., e.g., Fv, Fab, or F(ab')2, which bind noncompetitively to monovalent antigenic epitopes on the same antigen.

SO U.S., 13 pp., Cont.-in-part of U. S. Ser. No. 926,566, abandoned.
CODEN: USXXAM

IN Chang, Tse Wen

L9 ANSWER 10 OF 39 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

TI Study on preparation and biodistribution of PEG-immunoliposomes with active carboxylic terminals.

AB AIM In order to accumula into its target specifically, immunoliposomes must possess two characteristics: specific target efficiency to its target cells and prolonged circulation in blood. A new type of **polyethylene glycol** (PEG)-immunoliposomes carrying monoclonal **antibodies** at the distal end of PEG chains should be developed. METHODS A dipalmitoylphosphatidylethanolamine (DPPE) derivative of PEG with carboxyl group (DPPE-PEG3000-COOH) was newly synthesized. Small unilamellar **liposomes** were prepared from egg phosphatidyl choline and cholesterol (5:4, mol/mol) containing 6 mol% DPPE-PEG3000-COOH using reverse-phase evaporation method followed with bath sonication. Monoclonal **antibody** of human bladder cancer cell (BDI-1), which is highly specific to human bladder cancer cell, was conjugated to PEG-**liposomes** as well as mouse IgG at the distal end of **polyethylene glycol** chain. Doxorubicin was entrapped into these immunoliposomes by remote (NH4)2SO4 gradient loading method. The specific targeting efficiency of these immunoliposomes was tested by cytotoxicity test in vitro, enzyme-linked immune sorbent assay (ELISA) and indirect fluorescent immunoassay. Its biodistribution was carried out in mice. RESULTS The specific targeting efficiency of BDI-1 immunoliposomes (BDI-1-IML) to EJ cells has been demonstrated, in contrast to the non-specific human colon carcinoma cells (LOVO). PEG-**liposomes** linked with mouse IgG (mouse-IgG-immunoliposomes, IgG-IML) displayed lower reticulo-endothelial systems (RES) uptake and longer circulation time than **liposomes** without PEG after intravenous injection. CONCLUSION The long circulation of these PEG-immunoliposomes in vivo, combined with its specific targeting efficiency demonstrated in vitro, guarantees the positive targeting efficiency of these immunoliposomes to its target carcinoma in vivo.
SO Yaoxue Xuebao, (November, 2000) Vol. 35, No. 11, pp. 854-859. print. ISSN: 0513-4870.
AU Zhang Yu-feng (1); Xie Shuo-sheng; Hou Xin-pu (1); Gao Xiang; Zhang Shuo (1); Chen Zu-shun

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L1	121508	S LIPOSOME#
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=> d l9 11-15 ti abs so

L9 ANSWER 11 OF 39 CAPLUS COPYRIGHT 2002 ACS
TI Drug delivery system with two-step targeting
AB The present invention relates to a drug delivery system with two-step targeting, which comprises a combination: (a) a lipid carrier provided with cell targeting agent(s) to target the drug delivery system to specific cells or tissues; and (b) a drug enclosed in said lipid carrier and provided with a DNA targeting agent to target the drug to the nuclei of specific target cells. Furthermore, the invention relates to a method of cancer therapy in which the above drug delivery system is administered to a cancer patient. The goal is to treat or analyze both large **tumor** masses as well as small **tumor** cell clusters and single spread **tumor** cells. According to the invention, drug uptake in **tumors** will be markedly increased at the same time as the interaction of the drug with healthy organs and tissues can be minimized. The invention gives potential to convert palliative into curative treatment.

SO PCT Int. Appl., 22 pp.
CODEN: PIXXD2

L9 ANSWER 12 OF 39 CAPLUS COPYRIGHT 2002 ACS
TI Sterically stabilized anti-idiotype immunoliposomes improve the therapeutic efficacy of doxorubicin in a murine B-cell lymphoma model
AB A **liposome** contg. diverse synthetic lipid derivs. of **polyethylene glycol** (PEG) results in smaller distribution vol. and longer circulation time in blood and, thus, may improve drug targeting. The characteristics and therapeutic efficacy of immunoliposomes with similar liposomal formulation have never been studied in lymphoma models. The authors have developed immunoliposomes conjugated with S5A8 monoclonal **antibody**, an anti-idiotype **antibody** to 38C13 murine B-cell lymphoma, and loaded them with doxorubicin using an ammonium sulfate gradient. Purified **antibodies** were covalently coupled to the termini of PEG on the surface of small unilamellar **liposomes**. Cell binding and internalization ability of these immunoliposomes were estd. by a fluorescence assay using a pH-sensitive fluorescent dye (HPTS). The in vitro cytotoxicity of doxorubicin encapsulated in immunoliposomes was greater for idiotype-pos. 38C13 cells than for the idiotype-neg. variant of this cell line. In syngeneic C3H/HeN mice, doxorubicin encapsulated in immunoliposomes exhibited a long circulation time and was more effective at prolonging survival of mice bearing 38C13 **tumor** than non-targeted liposomal doxorubicin or free doxorubicin plus empty immunoliposomes. The results demonstrate the superiority of targeted therapy with these immunoliposomes and its potential in lymphoma treatment.
SO Int. J. Cancer (1999), 80(5), 723-730
CODEN: IJCNAW; ISSN: 0020-7136

L9 ANSWER 13 OF 39 CAPLUS COPYRIGHT 2002 ACS
TI Passive targeting with liposomal drug carriers
AB Passive targeting with liposomal drug carrier systems was reviewed with 83 refs. The current status of the passive targeting by the polyethyleneglycol coated **liposome** (PEG-**liposome**) were described in this review. Newly developed **liposomes**, contg. either monosialoganglioside GM1 or amphipathic **polyethylene glycol** (PEG) derivs., are not readily taken up by the macrophages in the RES and hence stay in the circulation for a relatively long period of time. Particularly, PEG is useful because of its ease of prepn., relatively low cost, controllability of mol. wt. and link ability to lipids or protein including the **antibody** by a variety of methods as compared with GM1 mols. So many recent studies have focused on the use of **liposomes** with surface assocd. PEG. The presence of PEG reduces binding of serum protein, i.e. opsonins marking the **liposome** for clearance by MPS. Pharmacokinetic anal. and therapeutic studies with **tumor** bearing mice revealed that PEG-**liposomes** with an av. diam. of 100-200 nm were accumulated efficiently in **tumor** tissue. Due to the capillary permeability of the endothelial barrier in newly vascularized **tumors** is significantly greater than that of normal tissues, PEG-**liposomes** could extravasate from blood circulation to **tumor** tissue. Results from clin. studies with doxorubicin encapsulated into PEG-**liposomes**(DOXIL) in AIDS-related Kaposi's sarcoma revealed an increased therapeutic efficacy compared to free-drug. Solid **tumors** generally possess the following pathophysiol. characteristics: (a) hypervasculature, (b) incomplete vascular architecture, (c) secretion of vascular permeability factors that stimulate extravasation within the cancer, (d) little drainage(lack of lymph vessel) of macromols. and particles, which results in their long-term retention in **tumor** tissue. These characteristics of solid **tumors** are the basis of the so-called EPR effect(enhanced permeability and retention effect). Thus, the permeability of the endothelial barrier in newly vascularized **tumors** is increased compared with that of healthy tissues. PEG-**liposome** can take advantage of the EPR effect for efficient targeting binding in the **tumor**. The localization of PEG-**liposomes** into the

interstitial space betwe **tumor** cells by a process of extravasation from **tumor** vessels (EPR effect) was revealed by the electron microscopic observations. Immunoliposomes for the treatment of solid **tumor** should satisfy a no. of requirements aimed at max. targeting effect of immunoliposome administered systemically in the bloodstream. Antigen binding site of the **liposome**-conjugated **antibody** must be accessible for unperturbed interaction with antigen on the surface of target cells. The blood clearance of immunoliposomes must be minimized in comparison with rate of extravasation in the **tumor**. As described above, PEG-**liposomes** offer the development of immunoliposomes with both long survival times in circulation and target recognition being retained in vivo. A new type of long-circulating immunoliposome, which was PEG-immunoliposome attached **antibody** at the distal end of PEG chain, so called the pendant type immunoliposome, was designed. To assist extravasation, the **liposomes** were of uniform, small size (100-130 nm). Elimination of immunogenic effect of Fc portion and of the increased RES clearance through specific recognition by MPS cells carrying Fc receptor, was achieved by using Fab' fragment instead of the whole **antibody**. For the active targeting following the passive targeting to the solid **tumor** tissue, Fab' fragment of 21B2 **antibody** which is anti-human CEA or transferrin (TF) was conjugated to prepd. the pendant type immunoliposome(Fab'-PEG-LP or TF-PEG-ILP). Both immunoliposomes showed the low RES uptake and the long circulation time, and resulted in enhanced accumulation of the **liposomes** in the solid **tumor**. TF-PEG-LP could internalized into **tumor** cells with receptor mediated endocytosis following extravasation into **tumor** tissue. The pendant type immunoliposome can escape from the gaps between adjacent endothelial cells and openings at the vessel termini during **tumor** angiogenesis by passive convective transport much rather than ligand directed targeting. Targeting to **tumor** tissue with the pendant type immunoliposome is particularly important for many highly toxic anticancer drugs for cancer chemotherapy. An ultimate goal of pendant type immunoliposome is the incorporation of a fusogenic mol. that would induce fusion of **liposome** following their binding to the target cells or their internalization by endocytosis. Such liposomal formulations should be useful for endocytotic internalization of plasmid DNA and other bioactive materials.

SO Drug Delivery System (1999), 14(6), 433-447
CODEN: DDSYEI; ISSN: 0913-5006

L9 ANSWER 14 OF 39 CAPLUS COPYRIGHT 2002 ACS

TI Anti GD2-immunoliposome-mediated targeting of [125I] metaiodobenzylguanidine to neuroblastoma and melanoma cells in vitro

AB Patients with neuroblastoma (NB) are often refractory to metabolic radiotherapy with radioiodine-labeled metaiodobenzylguanidine (MIBG), generally due to low, if any, expression of the transporter mol. responsible for MIBG uptake in **tumor** cells. Delivery of anticancer drugs in sterically stabilized (**polyethylene glycol** (PEG)-contg.) immunoliposomes (SIL) is an emerging tool for the selective delivery of antitumor drugs to cells expressing specific antigens. By taking advantage of receptor-mediated endocytosis of targeted **liposomes**, the delivery of MIBG to NB cells may be enhanced, bypassing the requirement for a drug-specific membrane transporter. NB cells, as well as some neuroectoderma-derived cell lineages, such as melanoma cells, express frequently disialoganglioside GD2. This surface disialoganglioside can, therefore, be utilized as a target to selectively delivery MIBG-loaded SILs to these GD2-expressing cells. We thus explored the feasibility to encapsulate 125I-MIBG into anti-GD2 immunoliposomes and investigated the cellular uptake and metab. of SIL-MIBG compared to free MIBG in a panel of NB and melanoma cell lines in vitro. We successfully loaded free MIBG into stabilized **liposomes** and covalently coupled them to monoclonal anti-GD2 **antibodies**. The relative expression of MIBG-transporter and GD2 detd. the degree of MIBG uptake. Uptake of SIL-encapsulated MIBG by all cell lines was higher than that of free MIBG, the only exception being the highly transport-competent, GD2-neg. cell line SK-N-BE2c. Moreover,

successful incorporation MIBG in melanoma cells, which are inherently non competent in taking up the free drug, could be achieved by SIL-MIBG. Interestingly, the intracellular half-life of SIL-MIBG was significantly more prolonged than that of free MIBG in all NB cell lines, which reportedly cannot efficiently store free MIBG in subcellular compartments. The retention of SIL-MIBG by NB and melanoma cells was similar to that obsd. with free MIBG in highly storage-efficient pheochromocytoma (PC) cells. Thus, targeting GD2-pos. cells with specific MIBG-loaded immunoliposomes appears a novel strategy for **tumor** cell killing, regardless of their competence to specifically incorporate the free compd.

SO J. Liposome Res. (1999), 9(3), 367-385
CODEN: JLREE7; ISSN: 0898-2104

L9 ANSWER 15 OF 39 CAPLUS COPYRIGHT 2002 ACS

TI GD2-mediated melanoma cell targeting and cytotoxicity of **liposome** -entrapped fenretinide

AB Melanoma is a highly malignant and increasingly common neoplasm. Because metastatic melanoma remains incurable, new treatment approaches are needed. Immunoliposomes have been previously shown to enhance the selective localization of immunoliposome-entrapped drugs to solid **tumors** with improvements in the therapeutic index of the drugs. Previously, we reported that the synthetic retinoid fenretinide (HPR) is an inducer of apoptosis in neuroblastoma (NB) cells, sharing the neuroectodermal origin with melanoma cells. HPR is a strong inducer of apoptosis also in melanoma cells, although at doses 10-fold higher than those achievable clin. Thus, our purpose was to investigate the in vitro potentiation of its cytotoxic effect on melanoma cells in combination with long-circulating GD2-targeted immunoliposomes. GD2 is a disialoganglioside extensively expressed on **tumors** of neuroectodermal origin, including melanoma. Murine anti-GD2 **antibody** (Ab) 14.G2a and its human/mouse chimeric variant ch14.18 have been ligated to sterically stabilized **liposomes** by covalent coupling of Ab to the **polyethylene glycol** (PEG) terminus. Ab-bearing **liposomes** showed specific, competitive binding to and uptake by various melanoma cell lines compared with **liposomes** bearing non-specific isotype-matched Abs or Ab-free **liposomes**. Cytotoxicity was evaluated after 2 h treatment, followed by extensive washing and 72 h incubation. This treatment protocol was designed to minimize non-specific adsorption of **liposomes** to the cells, while allowing for max. Ab-mediated binding. When melanoma cells were incubated with 30 .mu.M HPR entrapped in anti-GD2 **liposomes**, a significant redn. in cellular growth was obsd. compared to free HPR, entrapped HPR in Ab-free **liposomes** or empty **liposomes**. Cytotoxicity was not evident in **tumor** cell lines of other origins that did not express GD2. Growth of NB cells was also inhibited by immunoliposomes with entrapped HPR.

SO Int. J. Cancer (1999), 81(2), 268-274
CODEN: IJCNAW; ISSN: 0020-7136

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L9	39	DUPLICATE REM L8 (0 DUPLICATES REMOVED)

=> d l9 16-20 ti abs so au

L9 ANSWER 16 OF 39 MEDLINE
TI A combinatorial approach to producing sterically stabilized (Stealth) immunoliposomal drugs.
AB We have developed a method for producing sterically stabilized immunoliposomal drugs (SIL) readily applicable to a 'mix and match' combinatorial approach for the simple manufacture of a variety of ligand-targeted liposomal drugs. Ligands coupled to the terminus of **polyethylene glycol** (PEG) in micelles formed from PEG-lipid derivatives (mPEG2000-DSPE) could be transferred into preformed, drug-containing **liposomes** from the micelles in a temperature- and time-dependent manner. **Antibody** densities up to 100 microg **antibody**/micromol of phospholipid, and up to 3 mol% of mPEG2000-DSPE, could be simultaneously transferred from the ligand-coupled micelles into the liposomal outer monolayer with negligible drug leakage from **liposomes** during transfer and good stability in human plasma. Transfer of anti-CD19 into SIL resulted in a three-fold increase in binding of these **liposomes** to CD19+ human B cell lymphoma cells.
SO FEBS LETTERS, (1999 Oct 22) 460 (1) 129-33.
Journal code: EUH; 0155157. ISSN: 0014-5793.
AU Ishida T; Iden D L; Allen T M

L9 ANSWER 17 OF 39 CAPLUS COPYRIGHT 2002 ACS
TI Passive targeting to **tumor** tissue with **liposome**
AB A review with 14 refs. The current status of the passive targeting by the newly developed **polyethylene glycol**-coated **liposome** (PEG-**liposome**) is described in this review. **Liposomes** have demonstrated considerable promise as a carrier for the delivery of drugs in vivo. However, one of the drawbacks is that ordinary **liposomes** i.v. injected into animals are rapidly removed from the blood circulation by uptake primarily in the cells of reticuloendothelial system (RES). PEG-**liposomes** are not readily taken up by the macrophages in the RES and hence stay in the circulation for a relatively long period of time. PEG-**liposomes** are called STEALTH **liposomes**. Pharmacokinetic anal. and therapeutic studies with **tumor** bearing mice revealed that PEG-**liposomes** with an av. diam. of 100-200 nm were accumulated efficiently in **tumor** tissue. Due to the capillary permeability of the endothelial barrier in newly vascularized **tumors** is significantly greater than that of normal tissues, PEG-**liposomes** could extravasate from blood circulation to **tumor** tissue. Results from clin. studies with doxorubicin encapsulated into PEG-**liposomes** (DOXIL) in AIDS-related Kaposi's sarcoma revealed an increased therapeutic efficacy compared to free-drug. PEG-**liposomes** offer the development of immunoliposomes with both long survival times in circulation and target recognition being retained in vivo. A new type of long-circulating immunoliposome, which was PEG-immunoliposome attached **antibody** at the distal end of PEG chain, so called the pendant type immunoliposome, was designed. For targeting to the solid **tumor** tissue, Fab' fragment of the 21B2 **antibody** which is anti-human CEA or transferrin (TF) was conjugated to prepd. the pendant type immunoliposome (Fab'-PEG-LIP or TF-PEG-ILP, resp.). Both immunoliposomes showed the low RES uptake and the long circulation time, and resulted in enhanced accumulation of the **liposomes** in the solid **tumor**. TF-PEG-ILP could internalize into **tumor** cells with receptor mediated endocytosis following extravasation into **tumor** tissue. The pendant type immunoliposome can escape from the gaps between adjacent endothelial cells and openings at the vessel termini during **tumor** angiogenesis by passive convective transport much rather than ligand directed targeting. Targeting to **tumor** tissue with the pendant type immunoliposome is particularly important for many highly toxic anticancer drugs for cancer chemotherapy. An ultimate goal of pendant type immunoliposome is the incorporation of a fusogenic mol. that would induce fusion of **liposome** following their binding to the target cells or their internalization by endocytosis. Such liposomal formulations should be

useful for endocytotic internalization of plasmid DNA and other bioactive materials.

SO Drug Delivery Syst. (1999), 14(2), 79-85
CODEN: DDSYEI; ISSN: 0913-5006

AU Ishida, Osamu; Maruyama, Kazuo

L9 ANSWER 18 OF 39 CAPLUS COPYRIGHT 2002 ACS

TI Sterically stabilized anti-GM3, anti-Lex immunoliposomes: targeting to B16BL6, HRT-18 cancer cells

AB Various **tumor**-assocd. antigens have been identified as carbohydrates bound to lipids or to proteins expressed on **tumor** cell membranes. We prepd. **tumor**-specific immunoliposomes by coupling anticarbohydrate **antibodies**, such as antiganglioside GM3 **antibody** (DH2) or anti-Lex **antibody** (SHI), to **polyethylene glycol** (PEG)-coated **liposomes**. In vitro and in vivo targetability of anti-GM3 and anti-Lex immunoliposomes to B16BL6 mouse melanoma cells and HRT-18 human colorectal adenocarcinoma cells were monitored with a fluorescence microscopy, and analyzed by biodistribution assay of the immunoliposome in mice bearing the **tumor** tissues. The **antibody** coupling to the PEG **liposomes** did not greatly diminish the circulation time of the **liposome** in the C57BL/6 mouse model. In vitro cytotoxicity of doxorubicin encapsulated in **liposomes** was enhanced by **antibody** coupling, but still behind free doxorubicin. However, in vivo antitumor therapeutic efficacy of doxorubicin encapsulated in the immunoliposomes was far greater than the free drug or in conventional **liposomes**. Doxorubicin encapsulated in anti-GM3 immunoliposomes was able to reduce in vivo **tumor** growth and metastasis of B16BL6 mouse melanoma cells more greatly than any other formulations of the drug. This study suggests that **tumor**-assocd. antigens can be good target mols. for **tumor**-specific delivery of liposomal drugs or other synthetic drug delivery systems.

SO Oncology Research (1999), 11(1), 9-16
CODEN: ONREE8; ISSN: 0965-0407

AU Nam, Sang Min; Kim, Hong Sung; Ahn, Woong Shick; Park, Yong Serk

L9 ANSWER 19 OF 39 CAPLUS COPYRIGHT 2002 ACS

TI Immunopotentiating composition

AB The present invention provides an immunopotentiating compn. which comprises an antigen or antigen-inducing substance, and a carrier comprising a biocompatible material for effectively increasing an immune response derived from an antigen. The present invention further provides a method of producing an **antibody** by administering said immunopotentiating compn. to a mammal or bird, thereby modulating the immune response in said mammal or bird and recovering the **antibody** produced.

SO PCT Int. Appl., 80 pp.
CODEN: PIXXD2

IN Fujioka, Keiji; Sano, Akihiko; Nagahara, Shunji; Brandon, Malcolm Roy; Nash, Andrew Donald; Lofthouse, Shari

L9 ANSWER 20 OF 39 CAPLUS COPYRIGHT 2002 ACS

TI Cationic **liposome**:DNA complex vehicles encoding anti-angiogenic peptides for use in gene therapy

AB Cationic vehicles:DNA complexes comprising DNA encoding an anti-angiogenic peptide or DNA encoding a **tumor** suppressor protein and DNA encoding an anti-angiogenic peptide, as well as their use in gene therapy, are disclosed. The liposomal components may comprise 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine, 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine, and 2,3-dioleoyloxy(propyl-N,N,N-trimethylammonium chloride), optionally in combination with **polyethylene glycol** and a targeted ligand such as Arg-Gly-Asp, ferritin, or **antibodies** targeted toward HER2. DNA is prepd. encoding anti-angiogenic peptide fragments of thrombospondin I, fibronectin, laminin, platelet factor 4, angiostatin, and prolactin, as well as concatamers of these fragments. **Tumor** suppressor protein genes include p53, p21, or Rb. Thus, **liposome**:DNA vectors encoding

p53 in combination with hrombospondin I fragment reduc[redacted] **tumors** more effectively than p53 alone. The cationic polymer allows superior transfection of endothelial cells; Superfect is a better transfection agent than cationic **liposomes** for many different cell lines.
SO Eur. Pat. Appl., 47 pp.
CODEN: EPXXDW
IN Mixson, Archibald James

=> d l9 21-25 ti abs so au

L9 ANSWER 21 OF 39 CAPLUS COPYRIGHT 2002 ACS
TI Stealth-immuno-**liposomes**
AB A review with 6 refs. on **tumor**-targeting **liposomes** by modification of **liposome** surfaces with **polyethylene glycol** bound to Fab' fragment, etc.
SO Sogo Rinsho (1997), 46(9), 2294-2295
CODEN: SORIAX; ISSN: 0371-1900
AU Harashima, Hideyoshi; Kiwada, Hiroshi

L9 ANSWER 22 OF 39 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Receptor mediated delivery of daunomycin using immunoliposomes: Pharmacokinetics and tissue distribution in the rat.
AB Pharmacokinetics and tissue distribution of daunomycin and different liposomal formulations of daunomycin were determined. Special emphasis was thereby given to immunoliposome-mediated drug delivery. Three different types of 85 nm **liposomes** were used for this study: 1) conventional **liposomes**, 2) **liposomes** sterically stabilized with 2000 Dalton **polyethylene glycol** and 3) immunoliposomes prepared by coupling a control IgG-2a or monoclonal **antibody** to the distal end of the **polyethylene glycol** spacer. The **antibody** used was the OX26 monoclonal **antibody** to the rat transferrin receptor. Daunomycin and **liposomes** were administered by i.v. injection to the rat. Daunomycin and daunomycin in conventional **liposomes** were rapidly cleared from the plasma compartment. When compared to the free drug, daunomycin in conventional **liposomes** did accumulate to higher levels in liver and spleen and to lower levels in heart, lung and liver. In contrast, daunomycin in **liposomes** sterically stabilized with **polyethylene glycol** could not be detected in heart, lung, kidney, liver and spleen. Using nonspecific IgG-2a isotype immunoliposomes, tissue concentrations of immunoliposomes were reduced by at least a factor of two. Attachment of more than 29 OX26 monoclonal **antibodies** per **liposome** did not increase tissue levels in heart, kidney or lung. Tissue levels of OX26 immunoliposomes were reduced in all organs by coinjection of unbound OX26. In vitro, endocytosis of fluorescent immunoliposomes by RG2 rat glioma cells was observed. These data indicate that receptor mediated drug delivery to different tissues can be achieved using OX26 conjugated immunoliposomes.
SO Journal of Pharmacology and Experimental Therapeutics, (1997) Vol. 282, No. 3, pp. 1541-1546.
ISSN: 0022-3565.
AU Huwyler, Jorg; Yang, Jing; Pardridge, William M. (1)

L9 ANSWER 23 OF 39 CAPLUS COPYRIGHT 2002 ACS
TI In vivo targeting of surface-modified **liposomes** to metastatically growing colon carcinoma cells and sinusoidal endothelial cells in the rat liver
AB We prepd. immunoliposomes by covalent coupling of a randomly thiolated monoclonal **antibody** against the rat colon adenocarcinoma cell line CC531 to MPB-PE on the outer surface of conventional as well as PEGylated **liposomes** of about 100-nm diam. We attempted to target these immunoliposomes in vivo to CC531 cells growing metastatically in the liver of syngeneic rats. Only when the immunoliposomes contained PEG-DSPE, did we observe, both with fluorescent and radioactive labels, accumulation of label in many, but not all, metastatic nodules. The fluorescent label concd. in scattered areas within the nodules. By means

of transmission electron microscopy, using colloidal gold particles as an encapsulated morphol. marker, we established that the large majority of the **tumor**-assocd. gold particles located in areas not contg. **tumor** cells. Most of the gold was detected in cells with a macrophage morphol. We tentatively ascribe this to either **tumor** morphol. or to the coupling procedure we applied for the prepn. of the immunoliposomes, or both. The random thiolation step of the **antibody** mol. conceivably allows for the exposure of the Fc portion of (part of) the **antibody** mols. so as to permit interaction with Fc receptors on the macrophages. Expts. with immunoliposomes prepd. either by coupling of the **antibody** specifically via its Fc portion or by using F(ab1)2 fragments are in progress. The crucial condition of liposomal longevity as in the above expts., where PEG-ylation of the immunoliposomes was necessary in order to achieve accumulation in the **tumor** area, by no means represents a general requirement for successful **liposome** targeting. We have shown that for efficient **liposome** targeting to a cell population which is readily accessible from the circulation, and has a high affinity for the **liposomes**, i.c. the hepatic sinusoidal endothelial cells, the presence of PEG chains may even be counter-productive.

SO J. Liposome Res. (1997), 7(4), 419-432
CODEN: JLREE7; ISSN: 0898-2104

AU Scherphof, Gerrit L.; Kamps, Jan A. A. M.; Koning, Gerben A.

L9 ANSWER 24 OF 39 CAPLUS COPYRIGHT 2002 ACS

TI Sterically Stabilized Anti-HER2 Immunoliposomes: Design and Targeting to Human Breast Cancer Cells in Vitro

AB **Liposomes** (70-100 nm) of 1-palmitoyl-2-oleoylphosphatidylcholine, cholesterol, and **polyethylene glycol** (PEG)-modified phosphatidylethanolamine (PEG-DSPE) were conjugated to Fab' fragments of a humanized recombinant MAb against the extracellular domain of HER2/neu to create sterically stabilized immunoliposomes (anti-HER2 SL) as a drug carrier targeting HER2-overexpressing cancers. Conjugation employed maleimide-terminated membrane-anchored spacers of two kinds: a short spacer, providing attachment of Fab' close to the **liposome** bilayer, or a long spacer, with Fab' attachment at the distal terminus of the PEG chain. Confocal microscopy and spectrofluorometry of HER2-overexpressing breast cancer cells incubated with fluorescently labeled anti-HER2 SL prepd. with either spacer showed binding of **liposomes** (8000-23 000 vesicles/cell) followed by endocytosis (rate const. ke = 0.012-0.033 min-1) via the coated-pit pathway, evidenced by intracellular acidification and colocalization with transferrin. Uptake of anti-HER2 immunoliposomes by breast cancer cells with low HER2 expression, or after preincubation of cells with free anti-HER2 Fab', was less than 0.2% and 4.3%, resp., of the uptake by HER2-overexpressing cells. Increasing PEG-DSPE content (up to 5.7 mol %) in anti-HER2-SL prepd. with the short spacer decreased **liposome**-cell binding affinity 60-100-fold, while ke decreased only 2-fold; however, when Fab' fragments were conjugated via a PEG spacer, both binding affinity and ke were unaffected by PEG-DSPE content. Cell binding and internalization of anti-HER2 immunoliposomes increased at higher surface d. of conjugated Fab' fragments, reaching plateaus at .apprx.40 Fab'/**liposome** for binding and .apprx.10-15 Fab'/**liposome** for internalization. Uptake of anti-HER2 immunoliposomes correlated with the cell surface d. of HER2 and significantly ($p < 0.005$) correlated with the antiproliferative effect of the targeting **antibody** but not with the total level of cellular HER2 expression. The results obtained were used to optimize in vivo preclin. studies of anti-HER2 SL loaded with antineoplastic drugs.

SO Biochemistry (1997), 36(1), 66-75
CODEN: BICHAW; ISSN: 0006-2960

AU Kirpotin, Dmitri; Park, John W.; Hong, Keelung; Zalipsky, Samuel; Li, Wen-Lu; Carter, Paul; Benz, Christopher C.; Papahadjopoulos, Demetrios

L9 ANSWER 25 OF 39 CAPLUS COPYRIGHT 2002 ACS

TI Solid **tumor** treatment method using antitumor agent-containing **liposomes** with PEG coating and surface-attached **antibody**

AB A method of administerin[redacted]n antitumor compd. to a subjec[redacted]s disclosed. The method includes administering **liposomes** having sizes predominantly in the range 0.05 to 0.12 .mu., and contg. an antitumor compd. in **liposome**-entrapped form, a surface coating of **polyethylene glycol** chains, at a surface concn. thereof sufficient to extend the blood circulation time of the **liposomes** severalfold over that of **liposomes** in the absence of such coating, and surface-attached **antibody** mols. effective to bind specifically to **tumor**-assocd. antigens present at the **tumor** site. One **liposome** compn. includes doxorubicin in entrapped form, and, on the **liposome** surface, a monoclonal **antibody** against highly proliferating cells in a lung squamous cell carcinoma.

SO U.S., 17 pp. Cont.-in-part of U.S. 5,213,804.
CODEN: USXXAM

IN Allen, Theresa M.; Martin, Francis J.

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(FILE 'HOME' ENTERED AT 09:29:30 ON 09 APR 2002)

FILE 'CAPLUS, MEDLINE, BIOSIS, CA' ENTERED AT 09:29:39 ON 09 APR 2002

L1	121508	S	LIPOSOME#
L2	1787339	S	ANTIBOD?
L3	1758451	S	TUMOR#
L4	10936	S	L1 AND L2
L5	1727	S	L3 AND L4
L6	800		DUPLICATE REM L5 (927 DUPLICATES REMOVED)
L7	164117	S	POLYETHYLENE (W) GLYCOL
L8	39	S	L6 AND L7
L9	39		DUPLICATE REM L8 (0 DUPLICATES REMOVED)

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